

## LIPIDS OF *Heracleum lehmannianum* SEEDS

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*The lipid complex of the seeds of Heracleum lehmannianum B. has been studied. The qualitative and quantitative compositions of the neutral lipids and the phospho- and glycolipids, and the fatty acid compositions of the acyl-containing classes of lipids have been determined. It has been shown that the neutral and the polar lipids differ considerably from one another with respect to the level of certain fatty acids. The position-species compositions of the TAGs and of the main components of the phospholipids have been calculated from the results of lipolytic hydrolysis.*

The cow parsnip *Heracleum lehmannianum B.* (fam. Umbelliferae) is a perennial wild-growing herbaceous plant of the mountain regions of Central Asia [1]. Continuing an investigation of plant lipids, we have studied the seed lipids of this plant. Neutral lipids (NLs) were obtained by steeping the dried and ground seeds with hexane, and essential oils by the method of [2].

The hexane extract consisted of a creamy yellow oily liquid. The yield of the extract was 30% of the weight of the seeds and that of the essential oil was 1.8%. The essential oil was a clear liquid with a peculiar sharp smell. According to TLC (systems 1 and 2), it consisted of seven components, among which substances with  $R_f$  0.55 and 0.43 predominated.

By TLC (systems 2-4), with the help of model specimens of plant lipids and specific characteristic reactions, six classes of NLs were detected in the hexane extract (Table 1). The quantitative compositions of the components of the NLs were determined by CC followed by the analytical preparative chromatography of narrow fractions (see Table 1). As was to be expected, the main lipid class quantitatively was that of the triacylglycerols (TAGs). Free sterols and triterpenols were detected in the products of the severe hydrolysis of sterol and triterpenol esters.

Polar lipids were isolated by Folch's method [3]. The qualitative and quantitative compositions of the PLs freed from impurities were determined by two-dimensional chromatography followed by the colorimetric determination of the phosphorus in the spots [4, 5]. Phospholipids were represented by eight components (see Table 1). The main ones quantitatively were phosphatidylcholines (PCs), phosphatidylinositols (PIs), and phosphatidylethanolamines (PEs).

The total glycolipids were isolated from the polar lipids with the aid of preparative TLC [6]. According to the TLC results, the GLs consisted of five components, among which the digalactosyldiglycerides (DGDGs) and sterol glycosides (SGs) predominated (see Table 1).

To characterize the acyl-containing class of lipids we determined their fatty acid (FA) compositions (Table 2). The fatty acid series included nine acids. In all the lipid classes other than the N-acyl-PEs the saturated acids were represented mainly by palmitic and stearic, while in the N-acyl-PEs the main acids were capric, lauric, and palmitic. Among the unsaturated acids, oleic and linoleic predominated. However, the free fatty acids (FFAs) contained considerable amounts of linolenic acid.

The NLs and PLs were more unsaturated than the GLs (see Table 2). In the NLs the sterol and triterpenol esters were distinguished by the greatest saturation, and the TAGs by the greatest unsaturation. The pancreatic lipolysis of the TGs showed that, as was to be expected, the central positions of the molecules were esterified mainly by oleic and linoleic acids. From these results, using Coleman's method as modified by Markman et al. [7], we have calculated the species composition of the TAGs (Table 3). Of the 64 species represented 43 were present in amounts of 0.2-1.0%, while the main species were OOO (18.7%) and OLO (8.9%). On summing the results in terms of the saturation (S) and unsaturation (U) of the fatty acid radicals we

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TABLE 1. Class Composition of the Lipids of *H. lehmannianum* Seeds

Class of lipids	Amount, % on the weight of the extract
Neutral lipids	
Hydrocarbons	0.1
Sterol and triterpenol esters	0.8
TAGs	96.6
FFAs	0.9
Triterpenols	0.4
Sterols	1.2
Phospholipids	
PCs	30.4
PIs	24.8
PEs	19.5
N-Acyl-PEs	12.2
N-Acyl-lyso-PEs	6.1
PGs	2.8
Lyso-PCs	2.4
Lyso-PIs	1.8
Glycolipids	
Sterol glycoside esters	12.3
MGDGs	19.4
Sterol glycosides	28.1
DGDGs	38.8
Unidentified GLs	1.4

TABLE 2. Fatty Acid Compositions of the Lipids of *H. lehmannianum* Seeds (% GLC)

Class of lipids	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	$\Sigma_s$	$\Sigma_u$
Total NLs	2.9	5.2	1.5	8.1	1.0	4.7	54.6	19.6	2.4	22.4	77.6
TAGs	2.8	3.2	1.0	8.6	0.8	3.7	57.5	20.6	1.8	19.3	80.7
sn-2-MAGs	0.4	0.6	1.3	1.5	1.0	0.6	62.6	29.5	2.5	4.4	95.6
Sterol and triterpenol											
esters	4.5	1.8	2.7	50.6	1.9	5.3	21.5	11.7	–	64.9	35.1
FFAs	1.6	2.3	1.7	17.7	1.4	2.3	33.5	26.2	13.3	25.6	74.4
Total PLs	0.5	1.4	1.3	26.8	2.2	3.2	38.1	23.4	3.1	33.2	66.8
PCs	1.9	1.6	1.7	27.3	1.6	3.0	41.2	19.8	1.9	35.5	64.5
sn-1	3.8	3.7	4.5	52.5	2.5	6.8	15.6	10.6	–	71.3	28.7
sn-2	2.1	2.0	3.8	10.3	2.0	0.9	52.2	23.2	3.5	19.1	80.9
PIs	1.9	1.3	1.8	33.2	2.7	4.5	34.9	16.2	3.5	42.7	57.3
sn-1	2.6	2.0	2.2	60.8	1.6	6.9	15.5	8.4	–	74.5	25.5
sn-2	1.9	1.2	1.0	12.4	1.0	3.1	51.2	24.1	4.1	19.6	80.4
PEs	1.3	1.6	1.4	26.9	1.4	4.0	40.0	22.7	0.8	35.2	64.8
sn-1	2.1	2.4	2.0	50.2	1.0	9.8	17.4	15.1	–	66.5	33.5
sn-2	1.0	1.0	1.2	6.4	1.2	1.5	61.4	25.1	1.2	11.1	88.9
N-Acyl-PEs	4.0	5.6	6.3	26.3	2.5	4.9	32.6	16.9	0.9	47.1	52.9
N-Acyl-lyso-PEs	11.6	15.6	6.0	21.8	1.7	3.8	26.4	11.2	1.9	58.8	41.2
PGs	1.9	2.1	2.8	42.1	2.6	5.3	21.5	16.7	5.0	54.2	45.8
Lyso-PCs	2.1	2.4	2.9	48.7	1.0	5.1	21.3	16.5	–	61.2	38.8
Lyso-PIs	2.1	1.7	2.0	53.8	2.5	7.8	20.4	9.7	Tr.	67.4	32.6
Total GLs	7.5	5.8	4.1	40.0	5.3	8.5	18.1	9.0	1.7	65.9	34.1

obtained the following position-type composition of the TAGs of the cow parsnip *H. lehmannianum* (%): SSSem — 0; SUS — 7.1; SUU — 42.8; UUU — 48.5; SSU — 0.6; USU — 1.0.

In the PLs, the main components (PCs, PIs, and PEs) were more unsaturated than the minor components. The position distributions of the FAs in the glycerol moieties of the molecules of these PLs were determined by enzymatic hydrolysis with phospholipase A2 (see Table 2). It can be seen that the sn-2 positions of the glycerol moieties of the PCs, PIs, and PEs were more than 80% enriched with unsaturated acids. A high specificity (88.9%) of the distribution of the FA radicals was observed in the PEs. From the position distributions of the FAs of the main fractions of the PLs we calculated [8] possible position-species compositions of the PCs, PIs, and PEs (Table 4): the PCs, PIs, and PEs of the *H. lehmannianum* seeds each contained about 72 of them, including some in amounts of less than 0.1%. In all the phospholipids the predominating species were the 16:0/18:1 and 16:0/18:2 types.

TABLE 3. Position-Species Composition of the TAGs of *H. lehmannianum* Seeds\*

TAGs	C Amount, %	TAGs	Amount, %
OPP+PPO	0.2	SLP	0.2
OPO	0.6	OLP	2.0
LPO+OPL	0.4	LLP	0.5
OPM† +MPO	0.3	MLP	0.4
POP	0.9	PLS	0.2
SOP	0.5	OLS	1.0
OOP	4.2	LLS	0.2
LOP	1.2	PLO	2.0
MOP	0.9	SLO	0.9
POS	0.5	OLO	8.9
SOS	0.2	LLO	2.5
OOS	2.0	LeLO	0.3
LOS	0.5	MLO	1.9
MOS	0.4	PLL	0.6
POO	4.2	SLL	0.3
SOO	2.0	OLL	2.6
OOO	18.7	LLL	0.7
LOO	5.4	MLL	0.5
LeOO	0.5	OLLe	0.2
MOO	4.1	PLM	0.4
POL	1.2	SLM	0.2
SOL	0.6	OLM	1.9
OOL	5.5	LLM	0.6
LOL	1.5	MLM	0.4
MOL	1.2	OLeP	0.2
OOLe	0.5	PLeO	0.2
POM	0.9	OLeO	0.9
SOM	0.4	LLeO	0.2
OOM	4.1	MLeO	0.2
LOM	1.2	OLeL	0.2
MOM	0.9	OLeM	0.2
PLP	0.5	MLS	0.2

\*The 21 species present in amounts of 0.1% and less are not included in the table

†M = 10:0 + 12:0 + 14:0.

TABLE 4. Position-Species Compositions of the PCs, PIs, and PEs of *H. lehmannianum* Seeds

Species	PCs	PIs	PEs	Species	PCs	PIs	PEs
16:0-10:0	1.1	1.2	0.5	16:0-18:0	0.5	1.9	0.8
18:1-10:0	0.3	0.3	0.2	18:1-18:0	0.2	0.4	0.3
18:2-10:0	0.2	0.2	0.2	18:2-18:0	-	0.3	0.2
16:0-12:0	1.1	0.8	0.5	10:0-18:1	1.9	1.3	1.3
18:1-12:0	0.3	0.2	0.2	12:0-18:1	1.9	1.2	1.5
18:2-12:0	0.2	-	0.2	14:0-18:1	2.3	1.2	1.2
14:0-14:0	0.2	-	-	16:0-18:1	27.5	31.1	30.8
16:0-14:0	2.2	0.6	0.6	16:1-18:1	1.3	0.8	0.6
18:0-14:0	0.2	-	-	18:0-18:1	3.6	3.5	6.0
18:1-14:0	0.5	0.2	0.3	18:1-18:1	8.2	8.0	10.7
18:2-14:0	0.4	-	0.2	18:2-18:1	5.5	4.1	0.3
10:0-16:0	0.4	0.4	-	10:0-18:2	0.9	0.6	0.5
12:0-16:0	0.4	0.3	0.2	12:0-18:2	0.8	0.5	0.6
14:0-16:0	0.5	0.3	-	14:0-18:2	1.0	0.5	0.5
16:0-16:0	5.5	7.5	3.2	16:0-18:2	12.2	14.6	12.6
16:1-16:0	0.3	0.2	-	16:1-18:2	0.6	0.4	0.2
18:0-16:0	0.6	0.9	0.6	18:0-18:2	1.6	1.7	2.5
18:1-16:0	1.6	1.8	1.1	18:1-18:2	3.6	3.7	4.4
18:2-16:0	1.0	1.0	1.0	18:2-18:2	2.5	2.1	3.8
16:0-16:1	1.1	0.6	0.6	10:0-18:3	-	0.2	-
18:1-16:1	0.3	0.2	0.3	14:0-18:3	0.2	-	-
18:2-16:1	0.2	-	0.2	16:0-18:3	1.8	2.5	0.6
10:0-18:0	-	0.2	-	18:0-18:3	0.2	0.2	-
18:1-18:3	0.6	0.6	0.3	18:2-18:3	0.4	0.3	0.2

\*Table does not include species amounting to 0.1% and less (21, 8, and 14, for the PCs, PEs, and PIs, respectively).

From the position-species compositions of the individual PLs we calculated their type composition:

	PCs	PIs	PEs
Disaturated (SS)	13.8	15.1	7.1
Saturated-unsaturated (SU)	57.5	59.6	59.3
Unsaturated-unsaturated (UU)	23.3	20.4	29.5
Unsaturated-saturated (US)	5.4	4.9	4.1

In all the components of the PLs the saturated-unsaturated and unsaturated-unsaturated types predominated. In the PEs the amount of disaturated types was roughly half as much as in the PCs and the PIs.

These results confirm once again that individual classes of PLs differ substantially from one another in fine structure, which is probably due to the specificity of their acylation in biosynthesis according to the different roles of individual compounds in the occurrence of cell processes.

Thus, the investigations performed have shown that the cow parsnip *Heracleum lehmannianum* contains a broad set of various classes of lipids and that the acyl-containing lipids differ appreciably from one another in their levels of individual fatty acids.

## EXPERIMENTAL

Solvents were purified by generally adopted methods [9]. For TLC we used Silufol and silica gel 5/40  $\mu\text{m}$  from Chemapol (Czechoslovakia). CC was conducted on Chemapol silica gel 100/160  $\mu\text{m}$  at a ratio of NLs and absorbent of 1:40. The spots of the NLs, PLs, and GLs were revealed as in [10]. Solvent systems: 1)  $\text{C}_7\text{H}_{16}-\text{C}_6\text{H}_6$  (9:1); 2)  $\text{C}_6\text{H}_{14}-\text{C}_4\text{H}_{10}\text{O}-\text{CH}_3\text{COOH}$  (70:30:1); 3)  $\text{C}_7\text{H}_{16}-\text{C}_2\text{H}_5\text{COCH}_3-\text{CH}_3\text{COOH}$  (43:7:1); 4)  $\text{C}_6\text{H}_{14}-\text{C}_4\text{H}_{10}\text{O}$  (3:2); 5)  $\text{CHCl}_3-\text{CH}_3\text{OH}-\text{CH}_3\text{OH}-\text{NH}_3$  (65:35:5); 6)  $\text{CHCl}_3-\text{CH}_3\text{OH}-\text{H}_2\text{O}$  (65:35:5); 7)  $\text{CHCl}_3-\text{CH}_3\text{OH}-\text{CH}_3\text{COCH}_3-\text{CH}_3\text{COOH}-\text{H}_2\text{O}$  (10:5:4:2:1).

GLC was performed as in [10].

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